Meeting of the States Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction

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Standing agenda item: review of developments in the field of science and technology related to the Convention

Advances in science and technology: Understanding pathogenicity and virulence

Submitted by the United States of America

I. Introduction

1. States Parties are halfway through the current intersessional programme, the first for which science and technology (S&T) relevant to the Convention is a standing agenda item. Fittingly, the S&T agenda item is an experiment, wherein Parties are exploring effective mechanisms for keeping up to date on relevant S&T advances. The United States considers this an important goal. To achieve it, Parties would benefit from information from recent scientific papers on **what** the relevant scientific knowledge and technologies are, **how** they are routinely used in laboratories, and the potential significance of recent discoveries and trends. To this end, we review below specific examples of research on pathogenicity and virulence from the past year. Empowered with this information, States Parties can more easily keep up to date, recognize truly game-changing scientific knowledge and technologies and, most importantly, grasp the challenges they pose to the implementation of this Convention. The United States of America delegation seeks feedback from States Parties regarding the clarity, relevance, and usefulness of this paper, as well as suggestions for improvement.

2. With regard to S&T developments, States Parties are perhaps most interested in their implications for the BWC. Acknowledging this, we discuss in section II the potential implications posed by recent research on pathogenicity and virulence. Section III seeks to define and explain the key concepts of pathogenicity, virulence and host-pathogen interactions in a manner that is technically correct but accessible to non-specialists. Sections IV and V each comprise two examples of recent scientific papers representing the two major types of pathogens: viruses and bacteria. Key technical terms appear in italics and are defined in the text or in footnotes.

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II. Implications

3. Perhaps the most significant trend in this area in recent years is our steadily advancing appreciation of the importance of host-pathogen interactions. That is, rather than understanding disease as simply a function of infection by a pathogen, studies have increasingly led to an understanding that disease is the result of a complex interaction between the pathogen and the host organism. This helps us to understand why one microbe may cause disease and another, very similar one does not produce any symptoms, or why a pathogen may make some people ill and not others.

4. Scientific research will continue to produce knowledge about host-pathogen interactions. New technologies combined with this knowledge will enable quicker and more detailed analyses of how these interactions lead to disease. Taken together, these S&T developments can reveal key interactions between host and pathogen that can be blocked in order to treat or prevent disease. For example, discovery of *virulence factors*[1] can guide vaccine and drug development. It is highly desirable to have vaccines and drugs that specifically block the effects of virulence factors, because they can ameliorate symptoms while fighting the pathogen.

5. As more is learned about host-pathogen interactions, it will become easier to more accurately predict the presence and effects of virulence factors in emerging pathogens based on sequence similarities or antibody cross-reactivity, as was done by researchers in the case of botulinum toxin Type H (Example 4 in Section V below). In some instances, for example with methicillin-resistant *Staphylococcus aureus* (MRSA), this predictive capability is already well advanced.[2] When outbreaks from new or re-emerging pathogens occur, knowledge gained from studies on the mechanisms and evolution of *pathogenesis*[3] should enable more rapid responses and development of countermeasures.

6. A capability to make predictions about the outcomes of host-pathogen interactions is inherently dual use knowledge. In a positive sense, such knowledge would improve and hasten development of vaccines, therapeutics, and diagnostics with increased specificity for virulence factors. Such knowledge could also enable mitigation of the host immune response, turning it up or down to minimize host damage. On the other hand, a predictive capability could theoretically be applied to the design of more virulent pathogens. While the topic of "designer pathogens" is disquieting, it is important to note that there are significant knowledge gaps – as well as technical hurdles – to their production. A very good example is the current difficulty in making predictions about which genes in a pathogen contribute not only to virulence, but also to viability, transmissibility to other hosts, and stability in the environment. While the *genomes*[4] of many pathogens have been sequenced, the functions of individual genes are frequently unknown or incompletely understood – though continued research is expected to fill these knowledge gaps over time.

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[1] Virulence factors are genetic, biochemical or structural features of a pathogen that enable disease (discussed in Section III).

[2] Laabei M, Recker M, Rudkin JK et al. (9 Apr 2014) Predicting the virulence of MRSA from its genome sequence. *Genome Research* 24:839-849, http://genome.cshlp.org/content/24/5/839.

[3] Pathogenesis refers to the chain of events that lead to disease.

[4] A genome is the complete set of genetic material within an organism or microorganism, including its genes.

III. Pathogenicity and Virulence are Determined by Pathogen and Host Factors

7. *Pathogenicity* and *virulence* are closely related terms that are sometimes used interchangeably. In the case of microorganisms, pathogenicity is the capacity to cause disease in a host. Virulence is the **degree** of damage that a pathogenic microorganism, or *pathogen*, causes in a host.

8. Certain attributes of pathogens play roles in determining the presence and extent of disease; these attributes are called *virulence factors*. However, a pathogen's virulence is determined not only by what virulence factors it contains, but is dependent on factors within the host as well. These *host factors* include genetic background, physical condition, and the status of the host immune system. For example, immune status is an important host factor; some microorganisms cause disease in immunocompromised[5] humans but not in healthy humans. Genetic background is another example: the human immunodeficiency virus (HIV) cannot establish chronic infection in humans possessing mutations in a single gene called CCR-5. In many, if not most cases, host factors that affect virulence remain unknown; one common example is herpes simplex virus 1, which for unknown reasons causes cold sores in some humans but not others, despite being highly contagious. Therefore, pathogenicity and virulence are determined by both pathogen and host factors.[6]

IV. Viral Pathogenesis and Virulence

Box 1: Two major viral mechanisms of pathogenesis

1. **By directly causing death of infected cells**: To complete their life cycles, viruses must gain entry into host cells, multiply inside those cells, and release new viruses to spread to other cells. To multiply, viruses hijack host cell machinery and deprive that cell of energy and materials necessary for survival. Release of newly replicated virus ultimately kills the host cell. If not stopped by an effective antiviral immune response or therapeutic agent, cell death may proceed to destroy tissues and eventually organs. This destruction can take place quickly (as when cells in the brain are destroyed by infection with encephalitis viruses) or slowly (as the liver is destroyed by persistent infection with hepatitis viruses).

2. **By stimulating host immune responses that destroy cells and tissues**: Some viruses cause hosts to mount strong immune responses that lead to cell death and tissue destruction more severe than damage caused by viral infection itself. For example, some influenza viruses quickly elicit severe inflammation (the first stage of an immune response) in the lungs that damages delicate cells essential for breathing. Even if such an immune response were effective at destroying the virus, the host may die from lung damage caused by the immune response itself.

9. **Example 1: Recent studies on virulence factors of influenza virus provide an example of current approaches to studying viral pathogenesis.** In June of this year,

[5] An immunocompromised host is one with an immune system that is impaired; this impairment may be caused by drugs, genetic deficiency, certain cancers, or chronic infections like HIV.

[6] For a thorough discussion of this topic, see Casadevall and Porofski's 2001 paper Host-Pathogen Interactions: The Attributes of Virulence, available at www.iid.montana.edu/faculty/hardy/documents/virulence_attributes_Casadevall.pdf.

Kawaoka and colleagues published a paper[7] in the journal *Cell Host & Microbe* that described experiments using *recombinant*[8] avian influenza viruses generated in the laboratory. Genes from known influenza viruses were chosen for their high degree of similarity with genes from the 1918 pandemic influenza virus and assembled into a new "1918-like" avian influenza virus. This 1918-like virus was used to infect ferrets – a commonly used animal model for the *in vivo*[9] study of influenza virus. The virulence of the 1918-like virus was studied by measuring weight loss and mortality of infected ferrets. (The sicker an animal becomes, the less likely it is to eat; therefore, weight loss is used as a proxy for degree of illness.) The 1918-like virus caused ferrets to lose an amount of weight intermediate between that caused by an unaltered strain of avian influenza virus and by the 1918 virus, leading to the conclusion that the artificial 1918-like strain is more pathogenic than unaltered avian influenza virus and more similar to the original 1918 virus. They also examined the lungs of infected ferrets – both the original 1918 virus and the 1918-like virus spread throughout lung tissues and damaged those tissues, unlike the unaltered avian virus. These and further experiments prompted the researchers to conclude that pandemic risk exists, since the genes similar to those from the 1918 virus circulate naturally and have the potential for enhancing virulence were they to recombine into a 1918-like virus in nature.

10. **Example 2: Recent review of molecular mechanisms by which varicella zoster virus causes two diseases in humans.** In a different approach published in February of this year, Zerboni and colleagues summarized models of the pathogenesis of varicella zoster virus (VZV), which causes chickenpox[10]. Like the herpesvirus that causes cold sores, VZV produces *latent* infection, in which the viruses lie dormant in nerve cells (neurons) and can later reactivate to cause another infection. Unlike, for example, influenza viruses, which only infect respiratory tissues, the life cycle of VZV plays out across a variety of tissues: first immune cells (called T cells) in the tonsils, then via the blood to the skin (where it causes pustules, or pox), then into neurons and sometimes back again to the skin (where it causes shingles, a painful skin rash). Scientists have developed a picture of how VZV manipulates three host cell types – T cells, skin cells and neurons – by exhaustively cataloging the effects that viral proteins exert on host proteins. To discover the effects of these proteins, scientists mutated the VZV genes encoding the proteins in order to make the proteins nonfunctional. Using this method, scientists discovered several mechanisms by which VZV proteins prolong infection, including: (i) promoting survival of infected T cells and skin cells, (ii) enabling VZV to go unrecognized by the immune system, and (iii) inducing "gene silencing" that enables the virus to go dormant in neurons.

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[7] Watanabe T, Zhong G, Russell CA et al. (11 Jun 2014) Circulating Avian Influenza Viruses Closely Related to the 1918 Virus Have Pandemic Potential, *Cell Host & Microbe* 15: 692-705, http://www.cell.com/cell-host-microbe/abstract/S1931-3128(14)00163-2.

[8] A recombinant virus comprises genes or gene fragments assembled from different sources.

[9] The term *in vivo*, Latin for "within the living," means occurring within a complete living system. Most commonly, *in vivo* experiments are experiments conducted in animals.

[10] Zerboni L, Sen N, Olivier SL et al. (10 Feb 2014) Molecular mechanisms of varicella zoster virus pathogenesis. *Nature Reviews Microbiology* 12:197-210, http://www.nature.com/nrmicro/journal/v12/n3/full/nrmicro3215.html.

V. Bacterial Pathogenesis and Virulence

Box 2: Two major bacterial mechanisms of pathogenesis

1. **By invasion of host cells and tissues**: Invasion requires contacting and sticking to cells, secreting invasive substances (called invasins) and avoiding the immune system while bacteria multiply. For example, tuberculosis, gangrene, and streptococcal infections result when the causative bacteria invade host cells and tissues.

2. **By producing toxins**: Some pathogenic bacteria secrete exotoxins (such as botulinum, diphtheria or cholera toxins) that act at a distance from the infected site in the body. Endotoxins are parts of bacterial cells to which hosts may have severe immune reactions when the bacteria die and disintegrate in the host. For example, fever and shock result when endotoxins within pathogenic strains of *E. coli* bacteria are released into the bloodstream – a disease state known as sepsis.

11. **Example 3: Recent studies of virulence factors contributing to persistent infections of *Mycobacterium tuberculosis*.** Advances in microbiology and genome sequencing technologies are increasingly applied along with classical approaches to answer basic questions in bacterial pathogenesis. For example, in studying the invasive pathogen *Mycobacterium tuberculosis* (Mtb), Cunningham-Bussel and colleagues sought to identify potential virulence factors in the pathogen's *genome* in order to understand the ability of Mtb to survive for long periods in human lungs.[11] This research suggested Mtb enzymes as virulence factors that allow survival within host cells under different *in vivo* conditions. Targeting these enzymes with drug candidates may provide an effective way to interfere with Mtb growth and persistence in host cells.

12. **Example 4: Recent characterization of a newly discovered botulinum toxin.** A new botulinum toxin (Type H) from a strain of *Clostridium botulinum* was characterized by Barash and Arnon[12] using a combination of classical immunology, *genome* sequencing and animal studies.[13] Immunological approaches indicated that antibodies to known botulinum toxin (types A through G) did not react with the new toxin; genome sequencing revealed its similarities and differences to other botulinum toxins; and animal studies demonstrated its extreme toxicity as well as the potential for vaccine development. The evidence presented by the researchers suggested that existing therapeutics and vaccines would not be effective against the novel Type H toxin.

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[11] Cunningham-Bussel A, Zhang T, Nathan CF (5 Nov 2013) Nitrite produced by *Mycobacterium tuberculosis* in human macrophages in physiologic oxygen impacts bacterial ATP consumption and gene expression, *PNAS* 110(45): E4256-E4265, http://www.pnas.org/content/110/45/E4256.full

[12] Barash, JR and Arnon, SS. (7 Oct 2013) A Novel Strain of Clostridium botulinum That Produces Type B and Type H Botulinum Toxins Journal of Infectious Diseases 209:183-191, http://jid.oxfordjournals.org/content/209/2/183.

[13] Hooper et al., 2014. J Infect Dis. 209:167 (DOI: 10.1093/infdis/jit528); Dover et al. 2014. 209:192-202 (DOI: 10.1093/infdis/jit450).